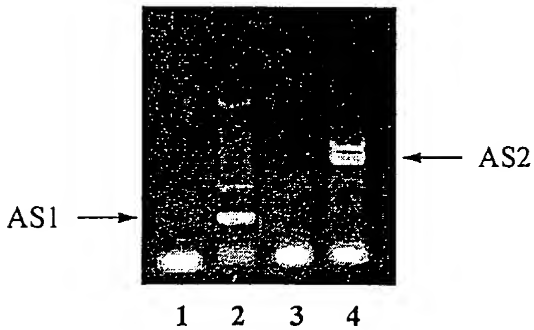
SERINE PROTEASE PRIMERS



1) Normal Ovary 2) Tumor
3) Normal Ovary 4) Tumor

Figure 1 shows a comparison of PCR products derived from normal and carcinoma cDNA as shown by staining in an agarose gel. Two distinct bands (lane 2) were present in the primer pair sense-His-antisense ASP-(AS1) and multiple bands of about 500 bp are noted in the carcinoma lane for the sense-His antisense-SER (AS2) primer pairs (lane 4).

```
             primer
201                                             250
   Prom  WVLTAAHCKK PNLQV....F LGKHNLRQRE SSQEQSSVVR AVIHPDY...  SEQ ID No:1
Tadg14   WVVTAAHCKK PKYTV....R LGDHSLQNKD GPEQEIPVVQ SIPHPCY...  SEQ ID No:2
   Try1  WVVSAGHCYK SRIQV....R LGEHNIEVLE GNEQFINAAK IIRHPQY...  SEQ ID No:3
   Scce  WVLTAAHCKM NEYTV....H LGSDTLGDRR A..QRIKASK SFRHPGY...  SEQ ID No:4
   Heps  WVLTAAHCFP ERNRVLSRWR VFAGAVAQAS PHGLQLGVQA VVYHGGYLPF  SEQ ID No:5

         251                                           300
   Prom  ...DAASHDQ DIMLLRLARP AKLSELIQPL PLERDCSA.. NTTSCHILGW
Tadg14   NSSDVEDHNH DLMLLQLRDQ ASLGSKVKPI SLADHCTQ.. PGQNCTVSGW
   Try1  ...DRKTLNN DIMLIKLSSR AVINARVSTI SLPTAPPA.. TGTKCLISGW
   Scce  ST...QTHVN DLMLVKLNSQ ARLSSMVKKV RLPSRCEP.. PGTTCTVSGW
   Heps  RDPNSEENSN DIALVHLSSP LPLTEYIQPV CLPAAGQALV DGKICTVTGW

         301                                           350
   Prom  GKTAD..GDF PDTIQCAYIH LVSREECEHA ..YPGQITQN MLCAGDEKYG
Tadg14   GTVTSPRENF PDTLNCAEVK IFPQKKCEDA ..YPGQITDG MVCAGSSK.G
   Try1  GNTASSGADY PDELQCLDAP VLSQAKCEAS ..YPGKITSN MFCVGFLEGG
   Scce  GTTTSPDVTF PSDLMCVDVK LISPQDCTKV ..YKDLLENS MLCAGIPDSK
   Heps  GNTQYYGQQ. AGVLQEARVP IISNDVCNGA DFYGNQIKPK MFCAGYPEGG

         351
   Prom  KDSCQGDSGG
Tadg14   ADTCQGDSGG
   Try1  KDSCQGDSGG
   Scce  KNACNGDSGG
   Heps  IDACQGDSGG
             primer
```

Figure 2. Comparison of amino acid sequence of TADG-14 with known serine protease catalytic domains.

OVER EXPRESSION OF TADG 14

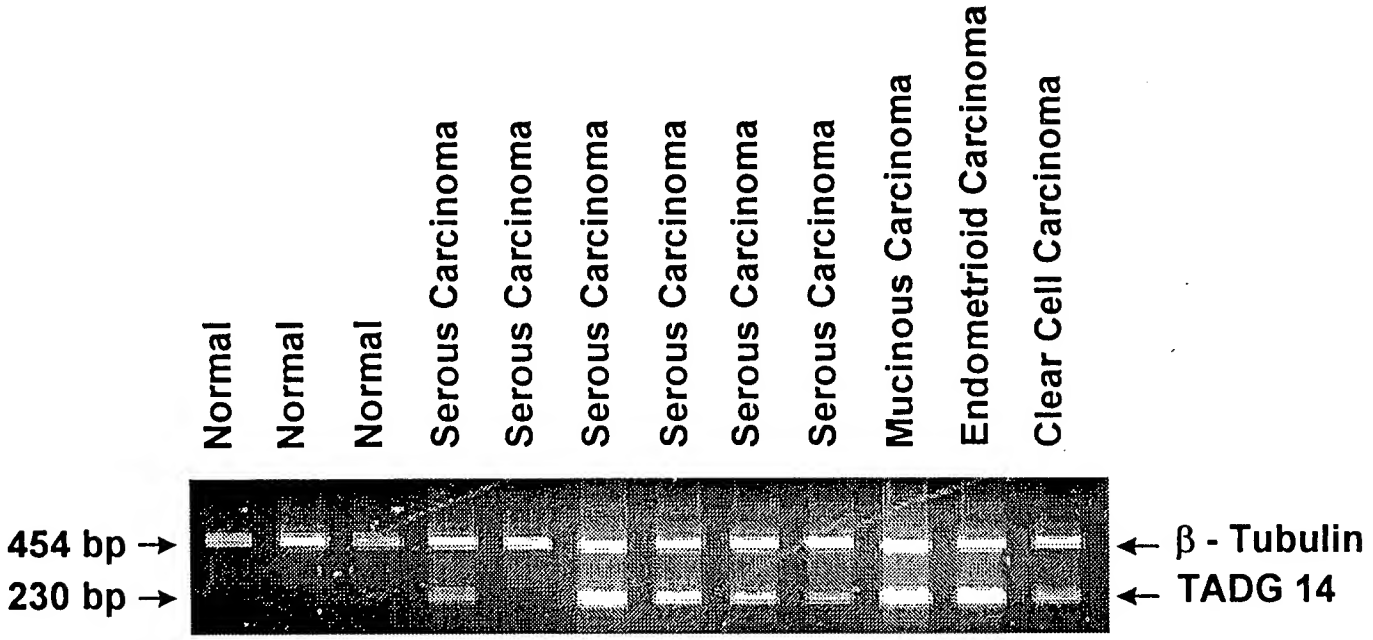


Figure 3. Overexpression of TADG-14 in ovarian carcinomas.

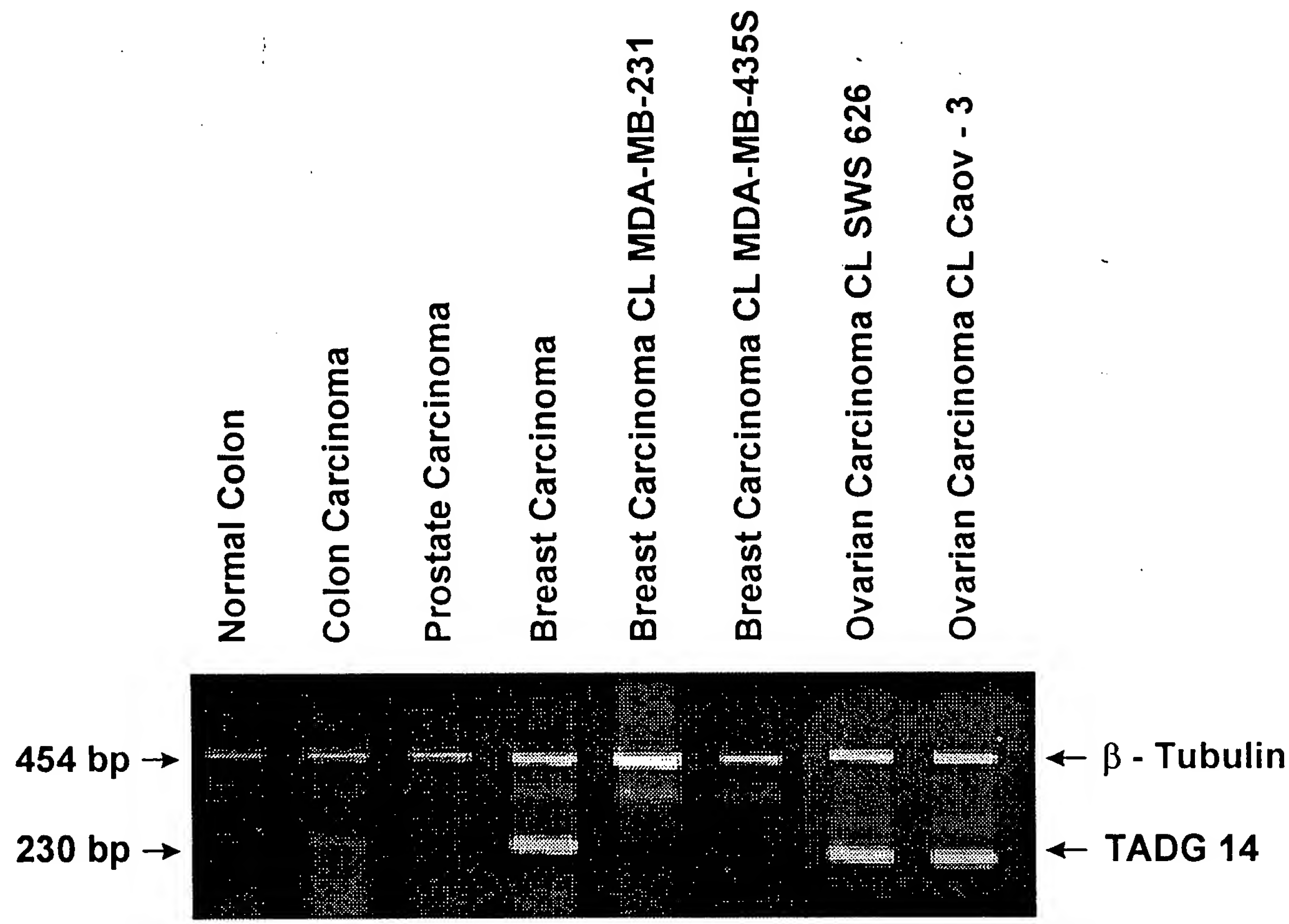


Figure 4. TADG-14 expression in tumors and cell lines.

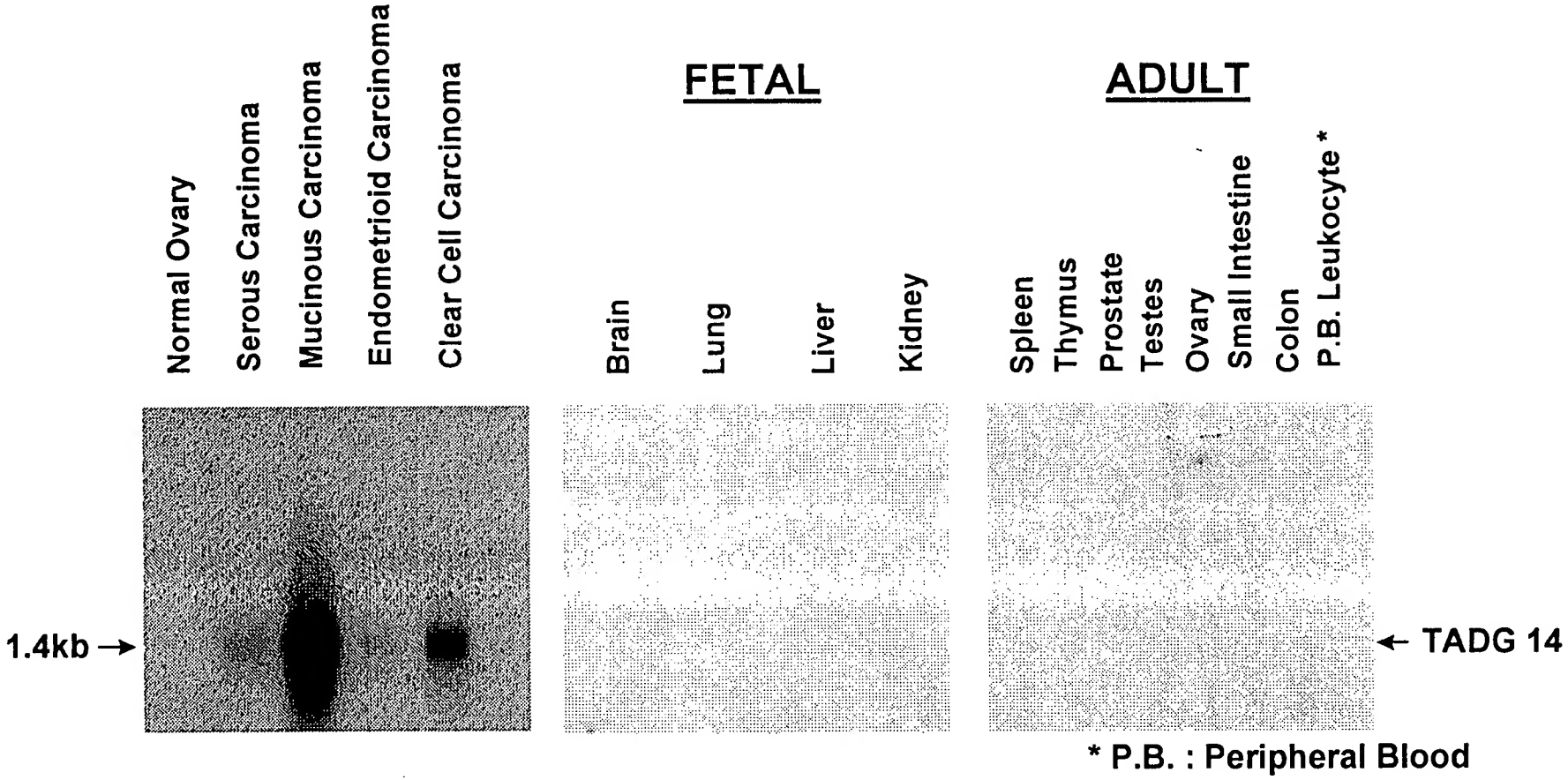


Figure 5. Blots: TADG-14 expression in fetal, adult and ovarian carcinoma tissues.

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121   GGATTATGAGGAATCCTTTCTATGTGATTTTCAATCATAGCAAGCAAGAAAGGCTCCAGT  180
181   GTCAAGGTAGTTCAGCTCTTACAGGATATAAAACAGTCCATACTTGAGAGAAAAAACTTA  240
241   GATCTGAGTGATGGAATGTGAAGCAAATCTTTCAAAATCAGTAGACATTTCTTGGACATA  300
301   AAACACAGATGAGGAAAGGGCTTCAAATTAGAAGTTACGTAATCACCATCAGAAAGTTCA  360
361   TGTTTGGTAAATTCTGTTACTAGAAATGTAGGAAATTCAGGTATAGCTTTGAATCCCAAT  420
421   TACACATTGGTCAGTGGGAAAACTAAGGGCCTCCAACAGGCAAATTCAGGGAGGATAGGT  480
481   TTCAGGGAATGCCCTGGATTCTGGAAGACCTCACCATGGGACGCCCCCGACCTCGTGCGG  540
                                         M  G  R  P  R  P  R  A  A -
541   CCAAGACGTGGATGTTCCTGCTCTTGCTGGGGGGAGCCTGGGCAGGACACTCCAGGGCAC  600
       K  T  W  M  F  L  L  L  L  G  G  A  W  A  G  H  S  R  A  Q -
601   AGGAGGACAAGGTGCTGGGGGGTCATGAGTGCCAACCCCATTCGCAGCCTTGGCAGGCGG  660
       E  D  K  V  L  G  G  H  E  C  Q  P  H  S  Q  P  W  Q  A  A -
661   CCTTGTTCCAGGGCCAGCAACTACTCTGTGGCGGTGTCCTTGTAGGTGGCAACTGGGTCC  720
       L  F  Q  G  Q  Q  L  L  C  G  G  V  L  V  G  G  N  W  V  L -
721   TTACAGCTGCCCACTGTAAAAAACCGAAATACACAGTACGCCTGGGAGACCACAGCCTAC  780
       T  A  A  H+ C  K  K  P  K  Y  T  V  R  L  G  D  H  S  L  Q -
781   AGAATAAAGATGGCCCAGAGCAAGAAATACCTGTGGTTCAGTCCATCCCACACCCCTGCT  840
       N  K  D  G  P  E  Q  E  I  P  V  V  Q  S  I  P  H  P  C  Y -
841   ACAACAGCAGCGATGTGGAGGACCACAACCATGATCTGATGCTTCTTCAACTGCGTGACC  900
       N  S  S  D  V  E  D  H  N  H  D+ L  M  L  L  Q  L  R  D  Q -
901   AGGCATCCCTGGGGTCCAAAGTGAAGCCCATCAGCCTGGCAGATCATTGCACCCAGCCTG  960
       A  S  L  G  S  K  V  K  P  I  S  L  A  D  H  C  T  Q  P  G -
961   GCCAGAAGTGCACCGTCTCAGGCTGGGGCACTGTCACCAGTCCCCGAGAGAATTTTCCTG 1020
       Q  K  C  T  V  S  G  W  G  T  V  T  S  P  R  E  N  F  P  D -
1021  ACACTCTCAACTGTGCAGAAGTAAAAATCTTTCCCCAGAAGAAGTGTGAGGATGCTTACC 1080
       T  L  N  C  A  E  V  K  I  F  P  Q  K  K  C  E  D  A  Y  P -
1081  CGGGGCAGATCACAGATGGCATGGTCTGTGCAGGCAGCAGCAAAGGGGCTGACACGTGCC 1140
       G  Q  I  T  D  G  M  V  C  A  G  S  S  K  G  A  D  T  C  Q -
1141  AGGGCGATTCTGGAGGCCCCCTGGTGTGTGATGGTGCACTCCAGGGCATCACATCCTGGG 1200
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1261  TGGACTGGATCAAGAAGATCATAGGCAGCAAGGGCTGATTCTAGGATAAGCACTAGATCT 1320
       D  W  I  K  K  I  I  G  S  K  G  *  SEQ ID NO:7
1321  CCCTTAATAAACTCACGGAATTC  SEQ ID NO:6
```

= Kozak's Consensus sequence

\+ = Conserved amino acids of catalytic triad H, D, S

NSS = Possible N - linked glycosylation site

= Poly - adenylation signal

= Conserved nt of catalytic triad

= aa required for formation of an oxyanion hole for catalytic activity

FLLL = Secretion signal sequence

Figure 6. Complete sequence of TADG-14 transcript including ORF and common domains.

Figure 7. Homology of TADG-14 with mouse neuropsin. 76% identity for ORF. Low homology outside of ORF.

Percent Similarity: 76.471   Percent Identity: 76.471

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      527 TTCTGCTTCTGTTCATGGGAGCGTGGCAGGGCTCACCAGAGCTCAGGGC 576
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      556 TCCTGCTCTTGCTGGGGGGAGCCTGGGCAGGACACTCCAGGGCACAGGAG 605
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      577 TCCAAGATCCTGGAAGGTCGAGAGTGTATACCCCACTCCCAGCCTTGGCA 626
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      606 GACAAGGTGCTGGGGGGTCATGAGTGCCAACCCCATTCGCAGCCTTGGCA 655
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      627 GGCAGCCTTGTTCCAGGGCGAGAGACTGATCTGTGGGGGTGTCCTGGTTG 676
          ||| ||||||||||||||| ||   |||  ||||||| |||||||| || |
      656 GGCGGCCTTGTTCCAGGGCCAGCAACTACTCTGTGGCGGTGTCCTTGTAG 705
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      677 GAGACAGATGGGTCCTCACGGCAGCCCACTGCAAAAAACAGAAGTACTCC 726
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      706 GTGGCAACTGGGTCCTTACAGCTGCCCACTGTAAAAAACCGAAATACACA 755
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      727 GTGCGTCTGGGTGATCATAGCCTCCAGAGCAGAGATCAGCCGGAGCAGGA 776
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      756 GTACGCCTGGGAGACCACAGCCTACAGAATAAAGATGGCCCAGAGCAAGA 805
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      777 GATCCAGGTGGCTCAGTCTATCCAGCATCCTTGCTACAACAACAGCAACC 826
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      827 CAGAAGATCACAGTCACGATATAATGCTCATTCGACTGCAGAACTCAGCA 876
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      856 TGGAGGACCACAACCATGATCTGATGCTTCTTCAACTGCGTGACCAGGCA 905
                   .         .         .         .         .
      877 AACCTCGGGGACAAGGTGAAGCCGGTCCAACTGGCCAATCTGTGTCCCAA 926
            ||| |||  ||| ||||||||  ||   |||||  |||  ||  || |
      906 TCCCTGGGGTCCAAAGTGAAGCCCATCAGCCTGGCAGATCATTGCACCCA 955
                   .         .         .         .         .
      927 AGTTGGCCAGAAGTGCATCATATCAGGCTGGGGCACTGTCACCAGCCCTC 976
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      956 GCCTGGCCAGAAGTGCACCGTCTCAGGCTGGGGCACTGTCACCAGTCCCC 1005
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      977 AAGAGAACTTTCCAAACACCCTCAACTGTGCGGAAGTGAAAATCTATTCC 1026
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1027 CAGAACAAGTGTGAGAGAGCCTATCCAGGGAAGATCACCGAGGGCATGGT 1076
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1056 CAGAAGAAGTGTGAGGATGCTTACCCGGGGCAGATCACAGATGGCATGGT 1105
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1077 CTGTGCTGGCAGCAGCAATGGAGCTGACACGTGCCAGGGTGACTCAGGAG 1126
     |||||| ||||||||||| || ||||||||||||||||| || || ||||
1106 CTGTGCAGGCAGCAGCAAAGGGGCTGACACGTGCCAGGGCGATTCTGGAG 1155
                  .         .         .         .         .
1127 GCCCTCTGGTGTGCGACGGGATGCTCCAGGGCATCACCTCATGGGGCTCA 1176
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1156 GCCCCCTGGTGTGTGATGGTGCACTCCAGGGCATCACATCCTGGGGCTCA 1205
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1177 GACCCCTGTGGGAAACCCGAGAAACCTGGAGTCTACACCAAAATCTGCCG 1226
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1227 CTACACTACCTGGATCAAGAAGACCATGGACAACAGGGACTGATCCTGG 1275
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08915659 .082197

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Neurop  1 MGRPPPCAIQPWILLLLFMGAWAGLTRAQGSKILEGRECIPHSQPWQAAL 50

         51 FQGQQLLCGGVLVGGNWVLTAAHCKKPKYTVRLGDHSLQNKDGPEQEIPV 100
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         51 FQGERLICGGVLVGDRWVLTAAHCKKQKYSVRLGDHSLQSRDQPEQEIQV 100

        101 VQSIPHPCYNSSDVEDHNHDLMLLQLRDQASLGSKVKPISLADHCTQPGQ 150
            ||| |||||.|. |||.||:||:.|.. |.|| |||||: ||. | . ||
        101 AQSIQHPCYNNSNPEDHSHDIMLIRLQNSANLGDKVKPVQLANLCPKVGQ 150

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        251 WIKKIIGSKG 260
            |||| . .:
        251 WIKKTMDNRD 260
```

Figure 8. Amino acid homology of TADG-14 with mouse neuropsin.